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Advancing disease monitoring of amyotrophic lateral sclerosis with the compound muscle action potential scan

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HIGHLIGHTS

- Compound muscle action potential scan-derived markers are sensitive to track disease progression in amyotrophic lateral sclerosis.
- Motor unit number estimation was most sensitive and may increase efficiency of clinical trials compared to clinical endpoints.
- Standardization of compound muscle action potential scans can further maximize its utility for clinical use and research.

ABSTRACT

Objective: To determine which compound muscle action potential (CMAP) scan-derived electrophysiological markers are most sensitive for monitoring disease progression in amyotrophic lateral sclerosis (ALS), and whether they hold value for clinical trials.
Methods: We used four independent patient cohorts to assess longitudinal patterns of a comprehensive set of electrophysiological markers including their association with the ALS functional rating scale (ALSFRS-R). Results were translated to trial sample size requirements.
Results: In 65 patients, 225 thenar CMAP scan recordings were obtained. Electrophysiological markers showed extensive variation in their longitudinal trajectories. Expressed as standard deviations per month, motor unit number estimation (MUNE) values declined by 0.09 (CI 0.07–0.12), D50, a measure that quantifies CMAP scan discontinuities, declined by 0.09 (CI 0.06–0.13) and maximum CMAP by 0.05 (CI 0.03–0.08). ALSFRS-R declined fastest (0.12, CI 0.08 – 0.15), however the between-patient variability was larger compared to electrophysiological markers, resulting in larger sample sizes. MUNE reduced the sample size by 19.1% (n = 388 vs n = 314) for a 6-month study compared to the ALSFRS-R.
Conclusions: CMAP scan-derived markers show promise in monitoring disease progression in ALS patients, where MUNE may be its most suitable derivate.
Significance: MUNE may increase clinical trial efficiency compared to clinical endpoints.

Abbreviations: A50, smallest size of the motor units making up the N50; ALS, amyotrophic lateral sclerosis; ALSFRS-R, ALS functional rating scale; BLUP, best linear unbiased prediction; CMAP, compound muscle action potential; D50, number of largest discontinuities required to elicit 50% of maximum CMAP; FMF, fine motor function; LME, linear mixed effects; MND, motor neuron disease; MU, motor unit; MUNE, motor unit number estimation; N50, number of largest MUs required to elicit 50% of the maximum CMAP; PMA, progressive muscular atrophy; PLS, primary lateral sclerosis; RR, relative range; S5, stimulus current required to elicit a target CMAP of 5% of the maximum CMAP; S50, stimulus current required to elicit a target CMAP of 50% of the maximum CMAP; S95, stimulus current required to elicit a target CMAP of 95% of the maximum CMAP.

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1. Introduction

Disease heterogeneity in amyotrophic lateral sclerosis (ALS) forms one of the major challenges to develop effective treatment (van Eijk et al., 2020a). Virtually all phase 2 and 3 clinical trials currently use the ALS functional rating scale-revised (ALSFRS-R) to evaluate efficacy of experimental therapies for ALS (van Eijk et al., 2020b). The ALSFRS-R, however, may be insensitive to detect subtle or early alterations in disease progression rate and could potentially miss effective treatments (Rutkove, 2015). More sensitive measures are, therefore, needed for ALS clinical trials (van den Berg et al., 2019).

Since ALS is characterized by the loss of motor neurons, methods that quantify motor neurons are promising tools to monitor disease progression. Prior to the clinical symptom onset, more than 50% of motor neurons innervating the muscle may already be lost (Emeryk-Szajewska et al., 1997, Hansen and Ballantyne, 1978, McComas et al., 1971). Quantifying the number of motor neurons may, therefore, pick up treatment effects earlier compared to clinical endpoints such as the ALSFRS-R. The number of motor neurons can be estimated by recording motor unit (MU) potentials over the muscle (McComas et al., 1971). Several electrophysiological methods, often referred to as motor unit number estimation (MUNE) methods, have been developed to monitor ALS disease progression (de Carvalho et al., 2018, Gooch et al., 2014). Nevertheless, mostly due to their laborious nature, required training and expertise, and relative invasiveness (de Carvalho et al., 2018), these methods have not yet routinely been implemented in clinical trials.

The electrophysiological muscle scan (MScan), or compound muscle action potential (CMAP) scan, has been suggested to mitigate drawbacks of earlier electrophysiological techniques. The CMAP scan is non-invasive (Drenthen et al., 2008, Drenthen et al., 2013, Garg et al., 2017, Henderson et al., 2006, Henderson et al., 2007, Henderson et al., 2009, Jacobsen et al., 2017, Maathuis et al., 2013, Sleutjes et al., 2014, Sleutjes et al., 2020), easy-to-apply, highly reproducible, well-tolerated and less labour intensive than various other MUNE methods (Jacobsen et al., 2017, Maathuis et al., 2011, Sleutjes et al., 2014). It has shown to be able to quantify disease progression in muscles affected by motor neuron disease (MND), (Baumann et al., 2012a, Baumann et al., 2012b, Henderson et al., 2007, Jacobsen et al., 2019, Maathuis et al., 2013) and to be related to functional decline (Jacobsen et al., 2019, Sirin et al., 2019) and survival (Baumann et al., 2012b). Though promising, it remains, however, to be established whether markers derived from CMAP scans hold value for clinical trials over clinical endpoints. The aim of this study is, therefore, to identify which electrophysiological markers are most sensitive to monitor disease progression and, second, to compare those markers to clinical endpoints in terms of sample size, using a multi-centre, well-defined cohort of patients with MND.

2. Methods

2.1. Study population, electrophysiological and clinical data

CMAP scan data for this study originated from four independent patient cohorts as part of previously performed studies executed over the past 15 years carried out in Australia, Denmark, Turkey, and the Netherlands (Baumann et al., 2012a, Jacobsen et al., 2019, Sirin et al., 2019, Sleutjes et al., 2016). All subjects gave informed consent to participate in these studies in accordance with the informed consent regulations of the institution where the research was conducted. Retrospective CMAP scan data comprised recorded stimulus currents and CMAP amplitudes from the same single thenar muscle within each patient tracked over time. Longitudinal patterns of the electrophysiological markers were assessed in patients with MND followed for a maximum duration of 15 months and with at least two visits.

With respect to the clinical endpoint, ALSFRS-R scores were obtained at the same day as the CMAP scan recordings. As the recordings were performed in the thenar muscles, which most closely reflects hand function, the longitudinal trajectories of both the ALSFRS-R and the fine motor function (FMF) subscore (sum of ALSFRS-R items 4–6) were assessed.

2.2. CMAP scan examination, analyses and extracted electrophysiological markers

The CMAP scan protocols are extensively described elsewhere (Jacobsen et al., 2018, Maathuis et al., 2013, Sleutjes et al., 2014). Briefly, the recordings were either performed using the CMAP scan-application on a Viking Select EMG system (Natus Neurology Incorporated, Inc., Middleton, WI, USA) or the MScan-application within the excitability software (Qtrac-S, institute of Neurology, Queen Square, London, UK). The median nerve was stimulated at the wrist and thenar CMAPs were recorded by surface electrodes in belly-tendon montage. Stimulus currents were delivered using decreasing currents from supramaximal to subthreshold levels. In general, the recordings took 10 minutes or less (Jacobsen et al., 2017, Sleutjes et al., 2020). We used two approaches to extract a comprehensive set of electrophysiological markers. First, we determined:

- MU number variables (MUNE - MU number estimation; N50 – Number of largest MUs required to elicit 50% of the maximum CMAP (Kristensen et al., 2019)),
- MU size variables (mean and largest unit size; A50 - Smallest size of the MUs making up N50 (Kristensen et al., 2019)).

These markers were obtained using the MScanFit tool (Bostock, 2016, Jacobsen et al., 2017) within the excitability software (Qtrac-P, institute of Neurology, Queen Square, London, UK). The MScanFit tool generates simulated CMAP scans by applying a mathematical model that includes MU number, MU sizes and threshold characteristics. These variables are subsequently optimized in an automated procedure to improve the fit between recorded and simulated scan using default optimization options (e.g. smallest MU size $\geq$ 25 μV and an initial relative spread of 2% for every MU (Bostock, 2016)). Secondly, we derived a set of electrophysiological markers directly from each scan:

- The maximum CMAP,
- Discontinuity variables (D50–Number of largest discontinuities required to elicit 50% of the maximum CMAP (Sleutjes et al., 2014); Step size at D50),
- Returner variables (Returners-Number of consecutive differences with increasing CMAPs at decreasing currents; Total returner size–summed size of returners (Sirin et al., 2019)),
- Threshold variables (S5, S50, and S95 - Stimulus currents required to elicit 5%, 50%, and 95% of the maximum CMAP; RR - Relative range (100* [S95 – S5]/S50)).

These markers were obtained using Matlab (R2018a: The MathWorks, Natick, Massachusetts, USA). The maximum CMAP and threshold variables were extracted from the CMAP scan using a moving average with a window-length based on a relative spread

of 2%. The discontinuity and returner variables were determined within the moving averaged minimum and maximum CMAP. The noise level for returners was set at 2% of the maximum CMAP (Sirin et al., 2019). The minimum detectable CMAP difference for discontinuities and returners was set at 25 μV. These constraints mitigated bias or variability by reducing the impact of noisy, low amplitude recordings, varying number of stimuli and outliers when assessing these markers.

2.3. Statistical analysis

Due to the right-skewed distribution of several electrophysiological markers, we either used square-root or log-transformed values. Linear mixed effects (LME) models were used to model the longitudinal trajectory of each outcome over time, where the fixed part contained a linear effect of time and the random part a random intercept and slope for time per individual. LME models can adequately take into account the varying frequency and timing of follow-up visits. We assessed non-linear relationships using smoothed B-splines (Kano et al., 2005).

In order to compare longitudinal characteristics between endpoints, we standardized ALSFRS-R, FMF and the electrophysiological markers. The standardized rate can be interpreted as the number of standard deviations change per month. We also evaluated the variability of the standardized rate of decline between the four cohorts and when categorizing patients by their site of onset in additional subgroup analyses. Furthermore, from each LME model we extracted the best linear unbiased prediction (BLUP) per individual, which reflects an individual's estimated rate of decline. BLUPs between the electrophysiological markers were subsequently compared by means of Pearson's r correlation coefficient.

Results were translated to trial design by calculating the required sample size for three clinical trial scenarios with follow-up durations of either 3, 6 or 12 months and a monthly, bimonthly or quarterly visiting scheme, respectively. Sample sizes for each scenario were calculated to detect a 30% reduction in disease progression rate with 80% power and a two-sided alpha of 5% according to (Ard et al., 2011). We used *R* (version 4.0.2) with Rstudio (version 1.1.463) for the statistical analyses. Linear mixed models were fitted using the lmer function and bootstrapped using the bootMer function (R-package lme4).

3. Results

3.1. Study population, electrophysiological, and clinical characteristics

A total of 225 CMAP scan recordings were obtained from the thenar muscles for the median nerve in 65 MND patients (left hand, n = 39; right hand, n = 26) from all four cohorts. Of those, three cohorts with a total of 56 patients had ALSFRS-R scores with a cumulative follow-up time of 319 months (Table 1). The median number of visits was 3 (range 2 to 7) with a median time interval between visits of 1.8 months (range 0.4 to 12.3). Nine CMAP scans were excluded due to movement artifacts (n = 4), high noise level (n = 1) and low maximum CMAP (<0.1 mV) (n = 4), resulting in 216 CMAP scans (96%) available for analysis. The baseline characteristics of the patient cohort are provided in Table 1. The age and ALSFRS-R at enrollment fall within the range compared to a large clinical trial population observed in the PRO-act database (Atassi et al., 2014). We had a slightly smaller percentage of patients with a limb onset (69% vs 76%).

An overview of the electrophysiological markers at baseline is provided in Table 2. At baseline the median number of stimuli was 471 (5th – 95th percentile: 305 – 690). Between the four cohorts, there was no difference at baseline for the maximum CMAP (p = 0.42) and MUNE (p = 0.48). With respect to the site of onset, the maximum CMAP at baseline showed no difference between patients with either bulbar, upper limb or lower limb onset. MUNE however was higher in bulbar onset patients compared to upper limb onset (MUNE = 64 vs MUNE = 32, p = 0.01). No difference was found between patients with bulbar and lower limb onset (MUNE = 64 vs. MUNE = 57, p = 0.61).

Table 1
Baseline characteristics obtained from all four cohorts with a total of 65 MND patients.

Patient characteristics	Total patient cohort (n = 65)*
Diagnosis, ALS/PMA/PLS	50/12/3
Age at enrollment, years	64 (37 – 78)
Sex, male	45 (69%)
Site of symptom onset, bulbar, upper limb, lower limb	20 (31%), 24 (37%), 21 (32%)
Symptom duration at enrollment, months	13.9 (4.4 – 66.6)
ALSFRS-R total score**	41 (24 – 47)
Fine motor function (subscore)**	10 (3 – 12)
ΔFRS, points per month***	−0.4 (-1.7 - −0.1)

ALS = amyotrophic lateral sclerosis, ALSFRS-R = ALS functional rating scale-revised, PMA = progressive muscular atrophy, PLS = primary lateral sclerosis. MND = motor neuron disease, *Unless indicated, data presented as median (5th – 95th percentile) or n (%), ** Initial score, available in three cohorts (n = 56), ***ΔFRS = (ALSFRS-R at first assessment – 48)/symptom duration since onset in months, available in three cohorts (n = 56).

Table 2
Baseline electrophysiological characteristics obtained from four cohorts with a total of 65 MND patients.

Electrophysiological markers	Unit	CMAP scan recordings (n = 65)
Maximum CMAP	mV	6.9 (1.1 – 12.2)
MUNE	-	51 (5 – 134)
N50	-	14 (1 – 41)
Mean MU size	μV	134 (56 – 373)
	%*	2.0 (0.7 – 19.3)
Largest MU size	μV	555 (195 – 1128)
	%*	8.4 (2.7 – 53.7)
A50	μV	170 (69 – 827)
	%*	2.3 (0.9 – 51.6)
D50	-	25 (3–54)
	%**	5.8 (0.7 – 10.4)
Step size at D50	μV	71 (28 – 313)
	%*	0.9 (0.5 – 10.7)
Returners	-	42 (13 – 70)
	%**	8.8 (3.4 – 18.4)
Total returner size	mV	12.3 (3.2 – 29.8)
	%*	205 (68–836)
S5***	mA	11.4 (6.2 – 26.1)
S50***	mA	15.3 (7.1 – 32.4)
S95***	mA	18.5 (9.1 – 42.2)
Relative range (RR)***	%	41.9 (21.6 – 71.2)

A50 = smallest size of the number of largest motor units required to elicit 50% of the maximum CMAP, CMAP = compound muscle action potential, D50 = Number of largest discontinuities required to elicit 50% of the maximum CMAP, MND = motor neuron disease, MU = motor unit, MUNE = motor unit number estimation, N50 = number of largest MUs required to elicit 50% of the maximum CMAP, RR = relative range, CMAP = compound muscle action potential, S5 = target CMAP to elicit 5% of the maximum CMAP, S50 = target CMAP to elicit 50% of the maximum CMAP, S95 = target CMAP to elicit 95% of the maximum CMAP. Data are median (5th – 95th percentile), *Normalized to maximum CMAP, ** Normalized to number of stimuli, *** Note that varying stimulus durations were applied (0.05, 0.1 and 0.2 ms)

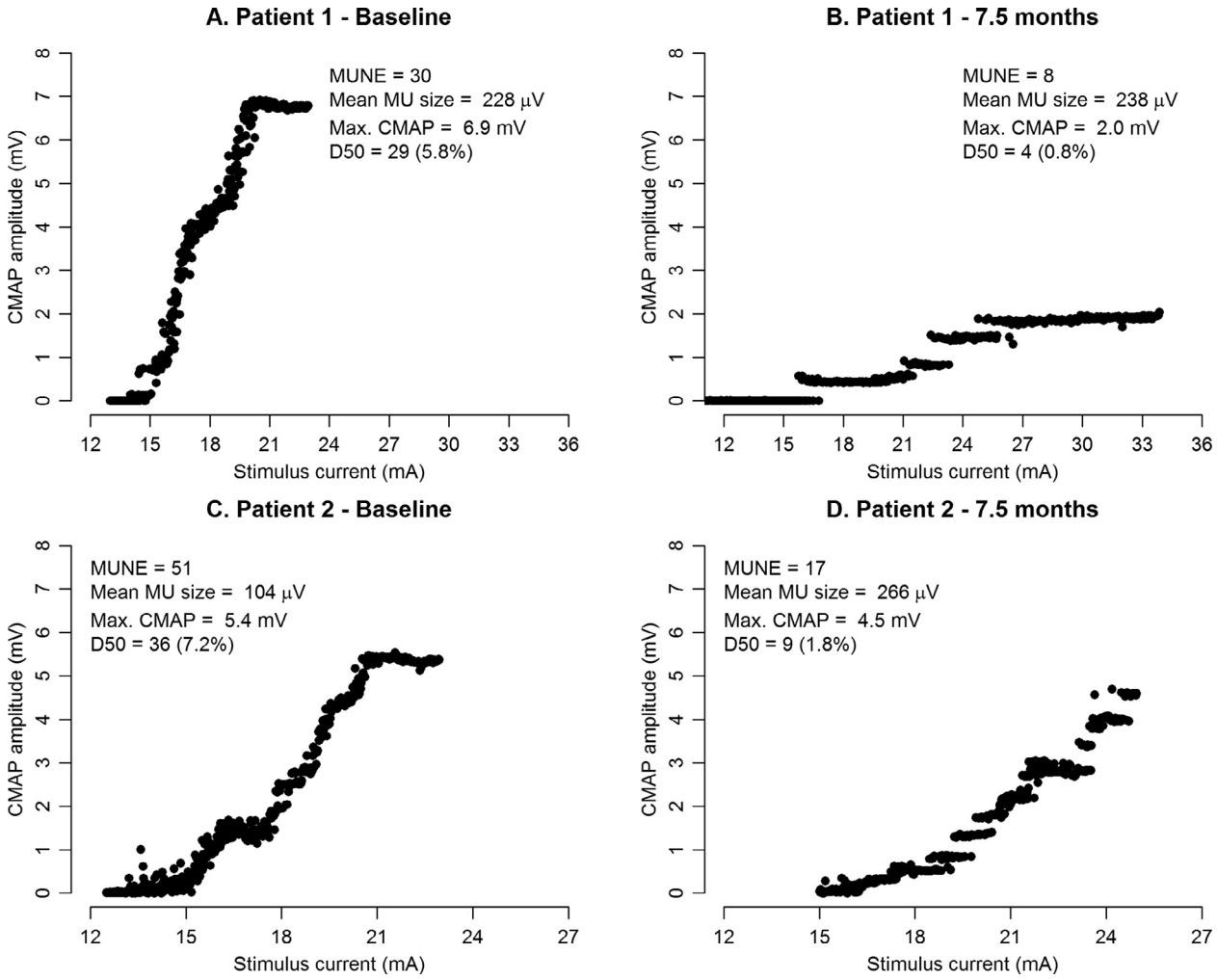

Fig. 1. Compound muscle action potential scans of two patients at baseline and after 7.5 months of follow-up. In patient 1 (top row), the mean motor unit (MU) size increased minimally over time from 228 μV to 238 μV. The compound muscle action potential (CMAP) amplitude however shows a dramatic drop, probably because reinnervation was less successful. Also D50 (absolute 29 to 4, normalized 5.8% to 0.8%) and motor unit number estimation (MUNE) (30 to 8) clearly decreased. In patient 2, the CMAP amplitude decreased minimally, whereas the mean unit size more than doubled from 104 μV to 266 μV. Also D50 (absolute 36 to 10, normalized 7.2% to 1.8%) and MUNE (51 to 17) dropped markedly. This indicates that, although MUs are lost, reinnervation was able to compensate for the effect on the CMAP amplitude.D50 = Number of largest discontinuities required to elicit 50% of the maximum CMAP.

3.2. Rate of decline and heterogeneity between electrophysiological markers in sensitivity to monitor disease progression

Fig. 1 illustrates in two patients the visual changes in CMAP scans during disease progression. The electrophysiological markers showed considerable variability in their longitudinal trajectories over time (Table 3). Where most declined over time, some markers showed an increase (e.g. related to MU sizes), and others remained relatively stable. Fig. 2 illustrates a few electrophysiological markers with a relative fast rate of decline ($p < 0.001$, Table 3) including MUNE (square-root rate of decline: −0.3/month, 95% CI: −0.4 - −0.2), maximum CMAP (rate of decline: −0.2 mV/month, 95% CI: −0.3 - −0.1), D50 (square rooted rate of decline: −0.2/month, 95% CI: −0.2 - −0.1), and normalized mean unit size (log rate of increase: 0.1%/month, 95% CI: 0.1 – 0.1). The rate of decline in MUNE values was strongly associated with the rate of decline in maximum CMAP (Pearson r = 0.61 (CI: 0.43 to 0.74), $p < 0.001$) and D50 (Pearson r = 0.62 (CI: 0.45 to 0.75), $p < 0.001$). Similarly, patients with a faster rate of decline on maximum CMAP had a faster decline in D50 (Pearson r = 0.65 (CI: 0.49 to 0.77), $p < 0.001$). Interestingly, patients with a faster rate of decline in MUNE values showed a larger increase in mean MU size (Pearson r = -0.62 (CI: −0.75 to −0.44), $p < 0.001$).

In subgroup analyses, we determined the standardized rates of decline in MUNE between the four cohorts showing large agreement with −0.10 (cohort 1), −0.09 (cohort 2), −0.10 (cohort 3), and −0.11 (cohort 4). Overall, the rate of decline was similar among the four cohorts ($p = 0.96$). In addition, when categorizing patients by their site of onset, the standardized rates of decline in MUNE were similar with −0.10 (bulbar), −0.08 (lower limb), and −0.11 (upper limb) ($p = 0.45$). We also compared our patient cohort (65 patients) with inclusion criteria of an ongoing platform trial (HEALEY ALS - Clinicaltrials.gov. ID: NCT04297683). When applying the three available inclusion criteria (symptom duration $\leq$ 36 months; age $\geq$ 18 years; sporadic/familial ALS according to revised El Escorial criteria) to our dataset, 35% (23 out of 65) would be excluded. This involved 11 patients with symptom duration > 36 months and 12 patients with PMA. In these 42 patients, the standardized rate of decline in MUNE was −0.11 (95% CI: −0.15 - −0.08), which resembles the standardized rate of decline of all 65 patients with −0.10 (95% CI: −0.12 - −0.08, Table 3).

Table 3
Overview of the standardized progression rates per month of the electrophysiological markers obtained from four cohorts with a total of 65 MND patients.

Electrophysiological markers	Unit	Standardized progression rates (SE)	P
Maximum CMAP	mV	−0.07 (0.01)	<0.001
MUNE	-	−0.10 (0.01)	<0.001
N50	-	−0.08 (0.01)	<0.001
Mean MU size	μV	0.05 (0.02)	0.03
	%*	0.10 (0.02)	<0.001
Largest MU size	μV	−0.01 (0.03)	0.39
	%*	0.08 (0.02)	<0.001
A50	μV	0.05 (0.02)	0.006
	%*	0.10 (0.01)	<0.001
D50	-	−0.09 (0.02)	<0.001
	%**	−0.10 (0.02)	<0.001
Step size at D50	μV	0.05 (0.02)	0.02
	%*	0.09 (0.02)	<0.001
Returners	-	−0.04 (0.02)	0.05
	%**	−0.02 (0.02)	0.15
Total returner size	mV	−0.08 (0.03)	0.003
	%*	0.02 (0.01)	0.04
S5***	mA	−0.01 (0.02)	0.39
S50***	mA	−0.01 (0.02)	0.23
S95***	mA	−0.02 (0.02)	0.14
Relative range (RR)***	%	−0.01 (0.03)	0.33

A50 = smallest size of the number of largest motor units required to elicit 50% of the maximum CMAP, CMAP = compound muscle action potential, D50 = Number of largest discontinuities required to elicit 50% of the maximum CMAP, MND = motor neuron disease, MU = motor unit, MUNE = motor unit number estimation, N50 = number of largest MUs required to elicit 50% of the maximum CMAP, RR = relative range, CMAP = compound muscle action potential, S5 = target CMAP to elicit 5% of the maximum CMAP, S50 = target CMAP to elicit 50% of the maximum CMAP, S95 = target CMAP to elicit 95% of the maximum CMAP. *Normalized to maximum CMAP, ** Normalized to number of stimuli, ***Note that varying stimulus durations were applied (0.05, 0.1 and 0.2 ms)

3.3. Rate of decline of ALSFRS-R over time and relation with MUNE

The ALSFRS-R (-0.9 points/month, 95% CI: −1.2 - −0.7, p < 0.001) and FMF subscores (-0.3 points/month, 95% CI: −0.4 - −0.2, p < 0.001) declined significantly over time in the 56 patients. The ALSFRS-R showed a standardized progression rate of −0.12 (95% CI: −0.15 - −0.08, p < 0.001) per month and FMF subscore of −0.08 (95% CI: −0.11 - −0.05, p < 0.001). The rate of decline for the ALSFRS-R fall within the range of other studies that include clinical trial populations of MND patients (de Jongh et al., 2021, van Eijk et al., 2019, van Eijk et al., 2018) and is in good agreement with the PRO-ACT database consisting of a large clinical trial population (Atassi et al., 2014). Fig. 3 shows the longitudinal relationship of the ALSFRS-R and FMF with MUNE. For FMF, and to a lesser extent for ALSFRS-R, a ceiling effect is visible when MUNE is large.

3.4. Trial design using electrophysiological markers derived from the CMAP scan

The electrophysiological markers with the fastest progression rates (see Table 3, with P < 0.001) based on the analysis of 65 patients (all four cohorts) were selected to determine their impact in trial design. In one cohort, no ALSFRS-R data was available. For the impact in trial design, we therefore proceeded with the other three cohorts (56 patients). Table 4 shows for three trial scenarios (3-month, 6-month, and 12-month trial) how sample sizes differ when the ALSFRS-R, FMF, MUNE, smallest size of the largest MUs, D50 and the maximum CMAP are taken as primary outcome. Progression rates were standardized to provide a direct comparison between endpoints and can be interpreted as number of standard deviations per month. In the 56 patients, these progression rates were −0.09 (95% CI: −0.12 - −0.07, p < 0.001) for MUNE, 0.10 (95% CI: 0.07–0.13, p < 0.001) for A50, −0.09 (95% CI: −0.13 - −0.06, p < 0.001) for D50 and −0.05 (95% CI: −0.08 - −0.03, p < 0.001) for the maximum CMAP. Due to the lower between-patient variability in rates of decline in CMAP scan-derived markers, sample sizes become relative smaller compared to the ALSFRS-R when follow-up time increases. At 6 months, for example, the sample size for MUNE was 19.1% smaller compared to the ALSFRS-R (n = 388 vs n = 314, Table 4). Compared to the FMF subscore, which more closely reflects the hand function, MUNE as outcome reduced the sample size by 35.7% (n = 488 vs n = 314, Table 4).

4. Discussion

The results of this study show that electrophysiological markers derived from the CMAP scan may hold value for monitoring disease progression in ALS clinical trials. The CMAP scan offers a set of markers sensitive to disease pathology (i.e. MU loss and/or enlarged MUs due to reinnervation) from a muscle generally affected in MND. Translated to trial design, the results indicate that MUNE and potentially other markers are promising candidate biomarkers for monitoring disease progression. This may be of significant value, as more sensitive endpoints are urgently needed to define early efficacy in phase 2 trials and improve the selection of compounds for phase 3. The CMAP scan may meet that requirement, where electrophysiological markers could provide early quantitative support for treatment effects.

Of the electrophysiological markers, MUNE, in terms of the required sample size for clinical trials, showed to be the most promising biomarker. Where most markers declined over time, various other markers associated with MU sizes increased when the disease progressed. Their progression rates accelerated when they were normalized to the maximum CMAP. As such, these markers likely reflect ongoing collateral reinnervation paralleled with progressive drop of the maximum CMAP due to denervation. Studying the dynamic interplay between denervation and reinnervation over time in further detail may therefore provide highly relevant pathological insights on the reinnervation capacity in MND patients. The largest discontinuities quantified by D50 also improved its performance in terms of the required sample size as outcome measure by normalization to the number of stimuli.

Given the retrospective study design for which we used data from previously conducted studies in patients with MND (Baumann et al., 2012a, Jacobsen et al., 2019, Sirin et al., 2019, Sleutjes et al., 2016), we are aware of the additional variability due to the different frequency and interval of follow-up visits, operators, electromyographic instrumentation, and recording and stimulation settings, which were independently applied in four centres with varying protocols based on published methodologies. The recordings further originated from a single muscle group (thenar) within one region (cervical) only. Although these suboptimal conditions could be regarded as limitations, the results of this study may actually provide support for the promising value of the CMAP scan for clinical trials. Adequate and standardized training for harmonisation of protocols have been shown to improve the quality of neurophysiological recordings (Neuwirth et al., 2018). Standardization of the applied equipment also helps to mitigate the variability between participating centres when conducting a multi-centre clinical trial. This may increase the sensitivity to monitor ALS disease progression, and aids in detecting subtler treatment effects. Similarly, obtaining CMAP scans from multiple muscles (Baumann et al., 2012b, Habeych et al., 2020, Higashihara et al., 2020, Kristensen et al., 2019, Sirin et al., 2019), may further enhance quantification of disease progression. This may also mitigate potential floor effects demonstrated by the

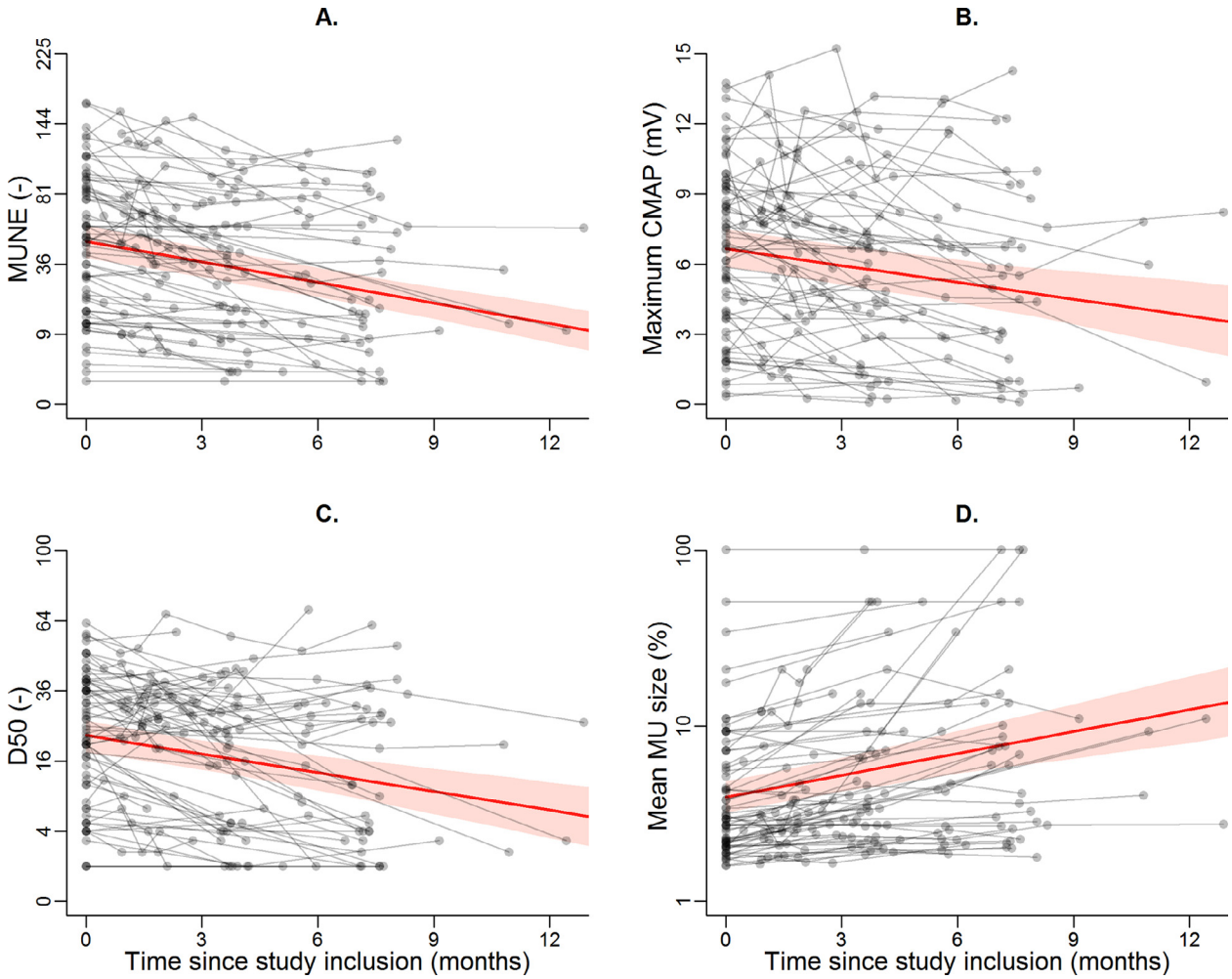

Fig. 2. Progression rates of various electrophysiological markers. Progression rates in (A) motor unit number estimation (MUNE), (B) maximum compound muscle action potential (CMAP), (C) D50, and (D) normalized mean motor unit (MU) size obtained from 65 patients. The red solid line is the mean progression rate over time and the red shaded area is the bootstrapped 95% confidence interval around the mean progression rate. Note that y-axes are square root (A, C) or log-transformed (D).D50 = Number of largest discontinuities required to elicit 50% of the maximum CMAP.

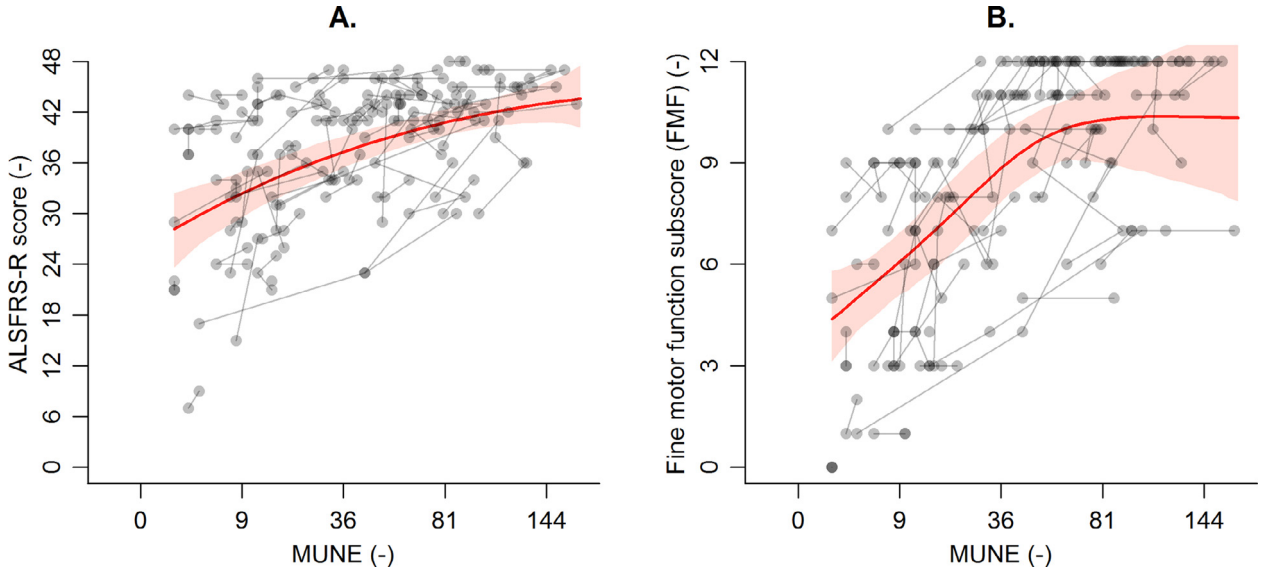

Fig. 3. Relation between motor unit number estimates and the ALS functional rating scale. The non-linear relation of motor unit number estimation (MUNE) values and (A) ALSFRS-R and (B) fine motor function (FMF) subscore. The red solid lines and the red shaded area is the 95% bootstrapped confidence intervals. Note that x-axes are square root transformed.

Table 4
Comparison of variance components and samples sizes for various electrophysiological endpoints with the ALSFRS-R obtained from three cohorts with a total of 56 MND patients.

Characteristic	ALSFRS-R	FMF	MUNE	A50	D50	Maximum CMAP
Monthly progression rate (SE)	−0.12 (0.02)	−0.08 (0.01)	−0.09 (0.01)	0.10 (0.02)	−0.09 (0.02)	−0.05 (0.01)
Between-patient variability ($\sigma^2_{Between}$)	0.12^2	0.07^2	0.08^2	0.09^2	0.10^2	0.06^2
Within-patient variability (σ^2_{Within})	0.19^2	0.25^2	0.19^2	0.23^2	0.31^2	0.30^2
Sample size estimates						
3-month				686		
6-month	522	1036	536	398	1154	2652
12-month	388	488	314	324	594	1028
	352	346	256		450	608

ALSFRS-R = ALS functional rating scale-revised, CMAP = compound muscle action potential, FMF = fine motor function, MND = motor neuron disease, MUNE = motor unit number estimation, A50 = smallest size of the number of largest motor units required to elicit 50% of the maximum CMAP, D50 = Number of largest discontinuities required to elicit 50% of the maximum CMAP. The three trial scenarios were based on visits every month for the 3-month trial, every two months for the 6-month trial and every three months for the 12-month trial. Sample sizes are calculated to detect a 30% reduction in progression rate with 80% power and a two-sided alpha of 5%. Note that monthly progression rates from Table 4 may slightly differ from Table 3 due to difference in number of patients/cohorts.

inability to record scans (observed in < 2%, 4 out of 225) or by low CMAP or MUNE values in severely wasted muscles. Hence, systematically investigating whether there are differences in the sensitivity to monitor disease progression in symptomatic or asymptomatic muscles may further provide insights on the robustness of CMAP scan-derived markers. This aids in providing relevant insights on how CMAP scan-derived markers develop in patients as the disease progresses and in determining the natural course from early to end stages of disease. Eventually, for the above, longitudinal prospective studies are required. Ultimately, one must be aware that only assessing in a representative trial population by implementation in future clinical trials that one can evaluate whether the CMAP scan can serve as surrogate outcome and that it truly reflects treatment effects. Prospective use may overcome potential regulatory hurdles generated by using the CMAP scan as efficacy endpoint. In this view, of importance is the currently ongoing phase 2 trial (Clinicaltrials.gov. ID: NCT04098406) where the CMAP scan is implemented as secondary outcome measure to evaluate whether a candidate drug slows ALS disease progression.

In the field of clinical trial design, it has been suggested to assess treatment effects by monitoring ALSFRS-R subscores separately to improve the efficiency of clinical trials (de Jongh et al., 2021, van Eijk et al., 2018). This creates other interesting possibilities by e.g. replacing specific subdomains with other biomarkers that may monitor the disease progression within this subdomain with a higher sensitivity. This study shows that electrophysiological markers may be optimal candidates as they require smaller sample sizes than the FMF subscore. Such a composite score may also more efficiently capture disease heterogeneity in patients with MND. However, this integrated approach will also pose other challenges for trial design and therefore require further investigations.

In conclusion, in our study we show the ability to monitor ALS disease progression using CMAP scan-derived electrophysiological markers. The CMAP scan is easy-to-apply and correlates strongly with functional outcomes. Of further relevance, the CMAP scan has taken an equivalently promising path in monitoring patients with spinal muscular atrophy (Kariyawasam et al., 2021, Sleutjes et al., 2020). Given the rapid developments of treatment options in this field, electrophysiological markers may aid in determining the best possible treatment tailored to the individual patient. Apart from biomarker performance, data complexity and collection in a multi-centre setting, there are multiple drivers that eventually determine widespread adoption (de Carvalho et al., 2018), where our study provides support that future studies are warranted to further maximize the utility of CMAP scans for clinical trials. This could eventually result in improved efficiency gains in trial design and early clinical decision making.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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